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TRANSMITTAL LETTER		DOCKET NUMBER: P-UC 5016	
SERIAL NO: 10/016,481	FILING DATE: November 1, 2001	EXAMINER: Unassigned	GROUP ART UNIT: 1645
INVENTION: PROKINETICIN POLYPEPTIDES, RELATED COMPOSITIONS AND METHODS			

TO COMMISSIONER FOR PATENTS

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner for Patents, Washington, D.C., 20231 on October 21, 2002.

By: Melanie K. Webster
Melanie K. Webster, Reg. No. 45,201

October 21, 2002
Date of Signature

Transmitted herewith are the following documents in connection with the above-identified application:

1. Request for corrected patent application publication with attached Exhibits A and B.

2. Return receipt postcard.

___ Please charge my Deposit Account No. 03-0370 the amount of $_____. A duplicate copy of this sheet is enclosed.

_X_ The Commissioner is hereby authorized to charge payment of any fees associated with this communication or credit any overpayment to Deposit Account No. 03-0370. A duplicate copy of this sheet is enclosed.

_X_ The Commissioner is hereby authorized to charge to Deposit Account No. 03-0370 any fees under 37 CFR 1.17 which may be required under 37 CFR 1.136(a)(3) for an extension of time in any concurrent or future reply requiring a petition for extension of time. A duplicate copy of this sheet is enclosed.

Respectfully submitted,

Melanie K. Webster

Melanie K. Webster
Registration No. 45,201
CAMPBELL & FLORES LLP
4370 La Jolla Village Drive
7th Floor
San Diego, California 92122
858-535-9001
USPTO CUSTOMER NO. 23601

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Our Docket: P-UC 5016

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Zhou and Ehlert	Group Art Unit: 1645
Serial No.: 10/016,481	Examiner: Unassigned
Filed: November 1, 2001	I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner for Patents, Washington, D.C., 20231 on October 21, 2002.
For: PROKINETICIN POLYPEPTIDES, RELATED COMPOSITIONS AND METHODS	By: Melanie K. Webster Melanie K. Webster, Reg. No. 45,201
Commissioner for Patents Washington, D.C. 20231	October 21, 2002 Date of Signature

REQUEST FOR CORRECTED PATENT APPLICATION PUBLICATION

Pursuant to 37 C.F.R. § 1.221(b), the publication of the above-identified application, publication No. US 2002/0115610 A1, published August 22, 2002, is respectfully requested to be corrected.

On page 14, Table 1, under the column entitled "Antagonistic Activity", please delete each of the three instances of "NO" and replace each with --ND--.

Submitted herewith is a copy of page 14 of the publication as it was published on August 22, 2002, with corrections noted thereon (Exhibit A). Also enclosed is a copy of page 58 as it was filed in the application on November 1, 2001, attached as Exhibit B, showing the correct wording in Table 1 as "ND".

Accordingly, Applicants request that this errors be corrected in the USPTO's electronic copy of the Specification and that the USPTO publish a corrected patent application publication.

No fee is deemed necessary to file this Request. If any fee is required, authorization is hereby given to charge the amount to Deposit Account No. 03-0370. A duplicate copy of this sheet is enclosed for this purpose.

Respectfully submitted,

October 21, 2002
Date

Melanie K. Webster
Registration No. 45,201
Telephone No.: (858) 535-9001
Facsimile No.: (858) 535-8949

CAMPBELL & FLORES LLP
4370 La Jolla Village Drive
Suite 700
San Diego, California 92122
USPTO CUSTOMER NO. 23601

lipase, and dickkopfs, a family of proteins that have an important role in early embryonic development.

[0155] A number of N-terminal substitution, deletion, and insertion mutants were constructed, and recombinant, refolded proteins produced. Bioassays with ileal smooth muscle preparations revealed that these mutant proteins at concentrations up to 250 nM are not able to elicit contractions (Table 1). However, an N-terminal deletion mutant (SEQ ID NO:16) and an N-terminal insertion mutant (SEQ ID NO:18) were able to weakly antagonize the contractile effect of prokineticin 1. Therefore, N-terminal variants of prokineticins, such as SEQ ID NOS:16 and 18, are potential therapeutics for inhibiting GI contractility.

TABLE 1

	Polypeptide	Contractile Activity	Antagonistic Activity
Wild Type (SEQ ID NO:3)	AVITGA [Prokineticin 1]	+	-
Insertion (SEQ ID NO:15)	GILAVITGA [Prokineticin 1]	-	ND
Deletion (SEQ ID NO:16)	VITGA [Prokineticin 1]	-	+
Substitution (SEQ ID NO:17)	AAAAAA [Prokineticin 1]	-	ND
Insertion (SEQ ID NO:18)	MAVITGA [Prokineticin 1]	-	+
Chimera	AVITGA [Co-lipase]	-	-
Chimera	AVITGA [dickkopf4]	-	ND
peptide (SEQ ID NO:19)	AVITGACERDVQCG	-	-
Cys mutation	AVITGA [Prokineticin 1] 18S	-	-
Cys mutation	AVITGA [Prokineticin 1] 60R	-	-

[0156] Chimeric recombinant proteins containing N-terminal sequences from prokineticin 1 and the C-terminal ten-cysteine domain from either colipase or Dickkopf 4 were also constructed. These two chimeric recombinant proteins were non-functional when tested with ileal smooth muscle preparation at concentrations up to 250 nM. Also tested was an N-terminal peptide (SEQ ID NO:19), which also was non-functional.

[0157] These results indicate that both N-terminal conserved sequence and C-terminal cysteine-rich domain are essential for the contractile activity of prokineticins.

Chimeric Prokineticins

[0158] A search of the draft human genome database with prokineticin cDNAs as queries revealed that genes encoding prokineticin 1 and 2 are composed of three exons. The signaling peptide and N-terminal conserved AVITG sequence are encoded in the first exon, while the cysteine-rich domain is encoded by exons 2 and 3. The 21 amino acid insertion of prokineticin 2 is encoded by an alternatively spliced mini-exon. To explore the functional difference of prokineticin 1 and 2, chimeric polypeptides were made with their exons 3 swapped (see FIG. 6). The chimeric polypeptides were designated chimera 12 (SEQ ID NO:13) and chimera 21 (SEQ ID NO:14), designating the swapped exons, as shown in FIG. 6.

[0159] Functional assays of refolded chimeric prokineticins 12 and 21 indicated that both of these chimeric polypeptides are active in contracting GI smooth muscle (FIG. 7A). However, the EC_{50} for the chimeric prokineticin 21 (SEQ ID NO:14) was about 8-fold higher than prokineticin 1 or prokineticin 2. Additionally, although the peak contractions were not affected, chimeric prokineticin polypeptides resulted in prolonged contraction of ileal strips (FIG. 7B). For wild type prokineticins, the time constants to midway contraction (half way from peak contraction to sustained plateau) were about 15 mins. In contrast, for the chimeric polypeptides, these time constants were prolonged to about 40 mins.

[0160] These results suggest that the chimeric prokineticins interact slightly differently with the receptor than wild type prokineticins, and cause less pronounced tachyphylaxis. Thus, the chimeric prokineticins (SEQ ID NOS:13 and 14) may have more potent pharmacological activity in vivo than wild-type prokineticins.

Effects of Prokineticin on Guinea Pig Ileum Smooth Muscle In Vivo.

[0161] To monitor the effects of prokineticin on the contraction of ileal smooth muscle in vivo, extraluminal force transducers were implanted on the serosal surface of the guinea pig ileum. Recombinant prokineticin 1 was then administered as a bolus into the jugular vein over a 10-second period. As shown in FIG. 8, an intravenous bolus of prokineticin 1 contracts guinea pig ileal smooth muscle in a dose-dependent manner. The threshold dose of prokineticin 1 is about 0.03 μg/kg, and a dose of 30 μg/kg produces the maximum effect.

[0162] Therefore, prokineticins, demonstrated above to be able to contract ileal smooth muscle in ex vivo preparations, are also effective in vivo.

Prokineticin Signal Transduction

[0163] To probe the potential signaling mechanisms of prokineticins, cell lines were identified that express prokineticin receptor endogenously. Over twenty cell lines were screened for binding to iodinated prokineticin 1. One cell line, M2A7 melanoma cells (ATCC CRL-2500; Cunningham et al., *Science* 255;325-327 (1992)), clearly displayed specific binding, with a receptor level of about 150 fmole/mg protein. Other cell lines that specifically bound prokineticin included M2 melanoma cells (Cunningham et al., *Science* 255;325-327 (1992)) and RC-4B/C pituitary tumor cells (ATCC CRL-1903). Cell lines that did not bind prokineticin included HEK293, COS-7, COS-1, Ltk-, NIH3T3, C6, NS10Y and HT-29 cells.

[0164] To assess signaling in M2A7 cells, cytosolic calcium was measured by fura-3 fluorescence using a FLIPR system (Fluorometric Imaging Plate Reader; Molecular Devices). Cells were suspended in HEPES medium and incubated with 2 μM of fura-3 AM for 20 min at 31° C. The cells were then centrifuged, washed, resuspended in fura-3-free medium and seeded into 96 wells at 4×10^4 cells per well. The cells were loaded with Fluo-3 AM (Molecular Probes) in standard buffer solution (130 mM NaCl,2 mM $CaCl_2$, 5 mM KCl, 10 mM glucose, 0.45 mM KH_2PO_4, 0.4 mM Na_2HPO_4, 8 mM $MgSO_4$, 4.2 mM $NaHCO_3$, 20 mM HEPES and 10 μM probenecid) with 0.1% fetal bovine serum for 1 h at 37° C., then washed with standard buffer solution. Transient changes in $[Ca_{2+}]_i$ evoked by prokineti-

contractile effect of prokineticin 1. Therefore, N-terminal variants of prokineticins, such as SEQ ID NOS:16 and 18, are potential therapeutics for inhibiting GI contractility.

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Chimeric recombinant proteins containing N-terminal sequences from prokineticin 1 and the C-terminal ten-cysteine domain from either colipase or Dickkopf 4 were also constructed. These two chimeric recombinant proteins were non-functional when tested with ileal smooth muscle preparation at concentrations up to